(a) **To explain and describe acid-base chemistry using the Henderson-Hasselbach equation.**

**Definition of acid and base:**
- “Bronsted-Lowry definition” is most commonly accepted in medicine:
  - Acid – Substance that donates a proton or hydrogen ion to another in solution
  - Base – Substance that accepts protons or hydrogen ions from another in solution

<table>
<thead>
<tr>
<th>Alternative definitions of acid-base include:</th>
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</thead>
<tbody>
<tr>
<td>- “Arrhenius definition”:</td>
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<tr>
<td>- Acid – Substance that dissociates in H₂O to produce H⁺</td>
</tr>
<tr>
<td>- Base – Substance that dissociates in H₂O to produce OH⁻</td>
</tr>
<tr>
<td>- “Lewis definition”:</td>
</tr>
<tr>
<td>- Acid – Substance that can accept an electron pair</td>
</tr>
<tr>
<td>- Base – Substance that can donate an electron pair</td>
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</table>

**Overview of Hydrogen ion (H⁺):**
- H⁺ is a hydrogen atom without its orbital electron → essentially a “proton”
- In aqueous solution, H⁺ is hydrated to form a “hydronium ion” (H₃O⁺):

\[
H^+ + H₂O \rightarrow H₃O^+
\]
- “H⁺ activity” (\(A_{H^+}\)) is a measure of how many H⁺ “seem” to be present in solution →

This is determined by:
- (i) Activity coefficient of H⁺ (g)
- (ii) Concentration of H⁺ ([H⁺]) – Quantity of H⁺ “actually” present in solution

\[
A_{H^+} = g \ [H^+]
\]

Note: \(A_{H^+}\) is synonymous with [H⁺] → because [H⁺] \(\approx\) \(A_{H^+}\)

**Overview of pH system:**
- pH is defined as the –ve base-10 logarithm of H⁺ activity (\(A_{H^+}\)) (where [H⁺] \(\approx\) \(A_{H^+}\)) → It serves as an indirect measure of H⁺ activity (and [H⁺]) in solution

\[
pH = -\log_{10} (A_{H^+}) \approx -\log_{10} [H^+]
\]
- pH and \(A_{H^+}\) (or [H⁺]) are inversely related in a non-linear fashion due to the log₁₀ scale (i.e. ↓ 1 unit pH = 10x ↑ \(A_{H^+}\) or [H⁺])
pH \ [H^+] \ (\text{nmol/L})

<table>
<thead>
<tr>
<th>pH</th>
<th>[H^+] (nmol/L)</th>
</tr>
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<tbody>
<tr>
<td>7.8</td>
<td>16</td>
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<tr>
<td>7.7</td>
<td>20</td>
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<tr>
<td>7.6</td>
<td>25</td>
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<td>7.5</td>
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<td>7.44</td>
<td>36</td>
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<td>7.4</td>
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<td>7.36</td>
<td>44</td>
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<td>7.3</td>
<td>50</td>
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<tr>
<td>7.2</td>
<td>62</td>
</tr>
<tr>
<td>7.1</td>
<td>80</td>
</tr>
<tr>
<td>7.0</td>
<td>100</td>
</tr>
</tbody>
</table>

- “Neutral pH” is the pH at which \([H^+] = [OH^-]\) → this is temperature dependent

Neutral point of H\(_2\)O is pH 7 at 25°C and 6.8 at 37°C (pH of neutral H\(_2\)O ↑0.017 unit for every 1 °C ↓ in temperature)

Overview of Dissociation Constant (K) and pKa:
- In solution, an acid (HA) will dissociate to a base (A\(^-\)) and H\(^+\):

\[
\begin{align*}
\text{HA} & \xrightleftharpoons[k_2]{k_1} \text{H}^+ + \text{A}^- \\
\text{rate of } & \text{HA} \rightarrow \text{H}^+ + \text{A}^- \quad \text{rate of } \text{H}^+ + \text{A}^- \rightarrow \text{HA}
\end{align*}
\]

\[
K = \frac{[\text{A}^-][\text{H}^+]}{[\text{HA}]}
\]

“Dissociation constant” (Ka) is the proportion of relative reactions present at equilibrium → Ratio of \(k_1:k_2\) (ie. \(K>1\) means \(k_1>k_2\) such that more HA dissociates into H\(^+\) and \(\text{A}^-\), than HA reassociates from H\(^+\) and \(\text{A}^-\))

- pKa is calculated as the –ve base-10 logarithm of the dissociation constant (K) of a substance → It is defined as the pH at which a substance is 50% dissociated (or ionised) in solution

\[
pKa = -\log_{10} (K)
\]

- pKa is an indirect measure of the extent of dissociation of the substance in solution (ie. \(\text{HA} \rightarrow \text{H}^+ + \text{A}^-\)) → in doing so, it determines its strength of acidity in solution

\[
\downarrow \text{pKa means } \uparrow \text{dissociation and stronger acid}
\]
\[
\uparrow \text{pKa means } \downarrow \text{dissociation and weaker acid}
\]

Henderson-Hasselbach Equation:

\[
\text{pH} = \text{pKa} + \log ([\text{A}^-]/[\text{HA}])
\]
This equation demonstrates that the ability of a substance to either donate a proton (i.e., act as an acid (HA)) or accept a proton (i.e., act as a base (A⁻)) depends on two factors:

- (i) pH of the solution
- (ii) pKa of the substance

**Derivation of Henderson-Hasselbach equation:**

\[
\begin{align*}
\text{HA} & \overset{k_1}{\underset{k_2}{\rightleftharpoons}} H^+ + A^- \\
\text{So,} \quad \frac{k_1}{k_2} &= \frac{[H^+][A^-]}{[\text{HA}]}, \\
\text{CH}_a \cdot k_1 &= k_2 \cdot [H^+] \cdot [A^-] \\
[H^+] &= \frac{k_2 \cdot [\text{HA}]}{[A^-]} = K \cdot \frac{[\text{HA}]}{[A^-]} \\
\log_{10} \text{both sides}, \text{ so:} \\
-\log_{10} [H^+] &= -\log_{10} K \cdot \frac{[\text{HA}]}{[A^-]} \\
\text{pH} &= \text{pK}_a + \log \frac{[A^-]}{[\text{HA}]} 
\end{align*}
\]
(b) To describe the chemistry of buffer mechanisms and to explain their relevant roles in the body.

(c) To describe the regulation of acid-base balance.

**H⁺ Balance in the Body:**

- **H⁺ production in the body:**
  - (1) “Volatile acids” (aka. “Respiratory acids”)
    - CO₂ is produced by [O] metabolism of carbohydrates and triglycerides (Ie. decarboxylation in TCA cycle) → Majority (75%) is hydrated in plasma to form Carbonic acid (H₂CO₃)
    - H₂CO₃ produces 15000 mmol H⁺/day
    - “Volatile acids” do NOT contribute to net acid balance in the body → because all H₂CO₃ in plasma is reformed as CO₂ in the lungs and eliminated
  - (2) “Non-volatile acids” (aka. “Metabolic acids” or “Fixed acids”)
    - (a) Lactate production
      - Produced from anaerobic metabolism of glucose and glycogen in RBC, skin and skeletal muscle → Produces 1500 mmol H⁺/day
      - Does not contribute to net acid balance in the body → because lactate is oxidised in the liver to regenerate HCO₃⁻ (via Cori cycle) → Exception is with excessive production (Ie. lactic acidosis with tissue hypoxia)
    - (b) Sulphuric acid
      - Produced from metabolism of S-containing a.a (esp Cys and Met) → Produces of 45 mmol H⁺/day
    - (c) Phosphoric acid
      - Produced from hydrolysis of phosphoproteins → Produces 13 mmol H⁺/day
    - (d) Other acids (Eg. HCl produced from a.a. metabolism, ketoacids produced from fat metabolism, Etc.) → Produces 12 mmol H⁺/day

**Note:**
- These acids are termed “Fixed acids” because they cannot be excreted by the lungs → Must be excreted by the kidneys (sulphuric acid, phosphoric acid and other acids) or metabolised by the liver (lactate)
- They contribute to net acid balance in the body → Normally produced at 1-1.5 mmol H⁺/kg/day (or ~ 70 mmol H⁺/day) → Thus, to maintain acid-base balance, these “fixed acids” must be completely excreted!

- **H⁺ excretion from the body:**
  - (1) Lungs
    - Eliminate all “volatile acids” (H₂CO₃) → 15000 mmol H⁺/day
  - (2) Liver
    - Eliminates “fixed acids” (mainly lactate) → 1500 mmol H⁺/day
  - (3) Kidney
    - Eliminates “fixed acids” (mainly phosphoric acid, sulphuric acid, other acids) as NH₄⁺ and “titratable acids”
    - Accounts for at least 70 mmol H⁺/day → 30 mmol/day as “titratable acids” and 40 mmol/day as NH₄⁺
Acid-base balance in the body:
- To maintain acid-base balance (and pH) in the body → Daily acid production must EQUAL daily acid excretion → So “Net acid balance” must equal ZERO

**Net acid balance (NAB) = Net acid production (NAP) – Net acid excretion (NAE)**

- Net acid production (NAP):
  - Normally ~ 70 mmol of H⁺ is produced daily from “fixed acids” (esp phosphoric acid, sulphuric acid, other acids)
- Net acid excretion (NAE):
  - Normally all “fixed acids” produced are excreted by the kidneys (~ 70 meq/day) → 30 mmol/day as “titratable acids” and 40 mmol/day as NH₄⁺
  - Almost all filtered HCO₃⁻ is reabsorbed → HCO₃⁻ excretion ~ 0 mmol/day

Overview of Acid-Base Homeostasis:
- [H⁺] in body fluid is precisely regulated → maintained at low plasma [ ] of 40 nmol/L (normal range 35-45 nmol/L) to keep extracellular pH ~ 7.4 (normal range pH 7.35-7.44) and intracellular pH ~ 6.8

Derangements of [H⁺] and pH can result in systemic effects (Eg. altered CNS reflexes, CVS depression, Etc.; See below) as a result of direct intracellular disturbances:
- (i) Altered protein function (Eg. enzyme activity, transporter activity, Etc.)
- (ii) Altered membrane excitability
- (iii) Disruption in metabolic pathways (esp energy production)
- (iv) Ion trapping of biological molecules in compartments, Etc.

This tight regulation of [H⁺] and pH is achieved by the following processes:
- (1) Buffering (1st line of defence) – Systems that immediately minimise changes in pH in the event that an acid or base is added to the body
- (2) Compensation (2nd line of defence) – Physiological processes that attempts to normalise pH by restoring the HCO₃⁻/PCO₂ ratio to normal → Either respiratory (rapid; mins-hrs) or renal (slow; hrs-days) → pH generally not completely restored to 7.4

<table>
<thead>
<tr>
<th>Process</th>
<th>H⁺ balance (mmol/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Production:</strong></td>
<td></td>
</tr>
<tr>
<td>CO₂ (as H₂CO₃)</td>
<td>15,000</td>
</tr>
<tr>
<td>Lactate</td>
<td>1500</td>
</tr>
<tr>
<td>Sulphuric acid</td>
<td>45</td>
</tr>
<tr>
<td>Phosphoric acid</td>
<td>13</td>
</tr>
<tr>
<td>Others (HCl, ketoacids)</td>
<td>12</td>
</tr>
<tr>
<td><strong>Output:</strong></td>
<td></td>
</tr>
<tr>
<td>Lungs</td>
<td>15,000</td>
</tr>
<tr>
<td>Liver</td>
<td>1500</td>
</tr>
<tr>
<td>NH₄⁺</td>
<td>40</td>
</tr>
<tr>
<td>Titratable acids</td>
<td>30</td>
</tr>
</tbody>
</table>
(3) Correction (3rd line of defence) – Mechanism that corrects acid-base derangement through correction of primary disorder

Acid-Base Homeostasis: Buffering

Overview of buffer systems:
- A “buffer” is a solution containing a weak acid and its conjugate base → Resists profound pH changes when exposed to a stronger acid or base by reversibly binding H⁺

\[
\text{Buffer} + H^+ \leftrightarrow H_\text{Buffer}
\]

- Effectiveness of a buffer is dependent on:
  - (i) Amount of buffer present → ↑ [buffer] ↑ the effectiveness of the buffer
  - (ii) Buffer’s pKa and pH of the carrier solution → Majority of buffering activity (80%) occurs within +/- 1 pH of the buffer’s pKa, with the maximal effect at its pKa (thus buffer effectiveness ↑ if buffer pKa and solution pH within +/- 1 unit)
  - (iii) “Open” (physiological) vs “closed” (chemical) buffer system → Buffer effectiveness ↑ with open systems

Buffering systems of the body:
- There are two types of buffer systems in body:
  - (1) Bicarbonate buffers (H₂CO₃-HCO₃⁻ buffer system) → Can only buffer “fixed/metabolic” acids (because it cannot buffer itself)
  - (2) Non-bicarbonate buffers (Hb, Phosphate and Protein buffer systems) → Can buffer both “respiratory” and “fixed/metabolic” acids

- Extracellular buffering:
  - (1) Blood – Mainly HCO₃⁻ and Hb (proteins and PO₄⁻ have minor roles)
    - (i) RBC – 1°ly Hb (35%; buffers 90% of H₂CO₃ and HCO₃⁻ (18%); proteins and PO₄⁻ are negligible
    - (ii) Plasma – 1°ly HCO₃⁻ (35%; buffers 70% of “metabolic” acids), protein (7%) and PO₄⁻ (2%)
  - (2) ISF – 1°ly HCO₃⁻ (ISF has ↑ capacity to buffer “metabolic” acids than blood because ISF volume (and HCO₃⁻ content) is 3x ↑ cf. blood)

- Intracellular buffering:
  - (i) 1°ly protein and PO₄³⁻ → because they occur at ↑ [ ] intracellularly and have pKa’s closer to intracellular pH (~ 6.8)
  - (ii) Fixed acid extrusion → Extrusion of IC H⁺ in exchange for a strong electrolyte (Na⁺, Cl⁻, lactate) via an anti-port transporter
  - (iii) Organellar buffering → Sequester or release H⁺ from IC organelle
  - (iv) Metabolic buffering → Alter production of acidic metabolites

“Isohydric principle” – All buffer systems in solution in the body that participate in defence of acid-base changes are in equilibrium with each other (i.e. changing pH will affect all buffer pair ratios in solution)

Bicarbonate-carbonic acid (HCO₃⁻-H₂CO₃) buffer system:
- Consists of H₂CO₃ (weak acid) and HCO₃⁻ salt (NaHCO₃ in ECF; KHCO₃ or Mg(HCO₃)₂ in ICF):

\[
\text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{H}_2\text{CO}_3 \leftrightarrow \text{H}^+ + \text{HCO}_3^-
\]

* Carbonic anhydrase (CA) is found in – (i) RBC, (ii) Alveolar walls, (iii) Renal tubular cells, and (iv) Renal PCT brush border
o In presence of strong acid – $H^+$ from acid buffered by $HCO_3^-$ → shifts to production of $CO_2$ production (via $H_2CO_3$) → $CO_2$ excreted from lungs
o In presence of strong base – Buffered by $H_2CO_3$ → Forms $HCO_3^-$ which is excreted from kidneys

- Most important buffer in ECF (RBC, plasma and ISF) → 80% buffering capacity:
  o It’s low pKa (6.1) relative to physiological pH and relatively small amounts in ECF ↓ its effectiveness as a buffer system in ECF
  o BUT this is offset by the fact that the system is “open” system → Can be controlled independently by the:
    - (i) Lungs – Excretion of $CO_2$ (or $H_2CO_3$) regulated rapidly by changes in minute ventilation
    - (ii) Kidneys – Excretion of $HCO_3^-$ and $H^+$ regulated more slowly

Note: Henderson-Hasselbach equation can be modified to relate the pH of blood to constituents of this buffer system:

$$pH = pKa + \log ([A^-]/[HA])$$

- $pKa$ of $H_2CO_3$ is 6.1
- $[H_2CO_3]$ cannot be measured in blood due to its rapid dissociation to $HCO_3^- + H^+$
  Thus, $[H_2CO_3]$ is estimated by amount of dissolved $CO_2$

$$[H_2CO_3] = \text{solubility coefficient of } CO_2 \times \text{PCO}_2 = 0.03 \times \text{PCO}_2$$

Thus: $$pH = 6.1 + \log ([HCO_3^-]/0.03x\text{PCO}_2)$$

**Haemoglobin (Hb) buffer system.**
- Hb is an intracellular protein in RBCs BUT acts as the major non-$HCO_3^-$ buffer in ECF
- Mechanisms for buffering capacity of Hb:
  o (1) ↑ [Hb] within RBCs (150 g/L) → Large amount of buffer present
  o (2) Structure of Hb
    - (a) Multiple (38) Histidine residues with Imidazole side chain (pKa 6.8) on globin chains of Hb → Anionic sites on Imidazole binds $H^+$ →
      Contributes to MAIN buffering capacity of Hb at physiological pH
    - (b) Amino acid groups on globin chains of Hb → $CO_2$ and $H_2CO_3$ can bind with terminal amino groups of amino acids of Hb to form “carbamino compounds”

$$HbNH_2 + CO_2 \rightarrow HbNHCOO^- + H^+$$
$$HbNH_2 + H_2CO_3 \rightarrow HbNH_3 + HCO_3^-$$

Nb. This accounts for 15% $CO_2$ transport in blood

- (3) Hb is a weak acid
  - Hb exists as weak acid (HHb) and salt (KHb) with a $pKa$ 6.8 → it is an even weaker acid cf. $HCO_3^- - H_2CO_3$ buffer system ($pKa$ 6.1)

$$HCl + KHb \rightleftharpoons HHb + KCl$$
$$\uparrow$$
$$H^+ + Hb^-$$

$$H_2CO_3 + KHb \rightleftharpoons HHb + KHCO_3$$
Thus, in presence of ECF acid $\rightarrow$ Hb buffers extracellular H$^+$ $\rightarrow$ leads to HCO$_3^-$ formation within RBC $\rightarrow$ HCO$_3^-$ accumulates and diffuses down its electrochemical gradient out of RBC $\rightarrow$ ↑ plasma HCO$_3^-$

- (4) RBC contains carbonic anhydrase and has ↑ solubility for CO$_2$:
  - Hb contributes to $>90\%$ of blood capacity to buffer CO$_2$ (as H$_2$CO$_3$)
  - Tissue CO$_2$ diffuses into RBC whereby it is hydrated to form H$_2$CO$_3$ by CA $\rightarrow$ (i) H$^+$ produced then preferentially binds DeoxyHb (to form Reduced Hb), thus allowing offloading of O$_2$ to tissues, and (ii) HCO$_3^-$ produced diffuses down its electrochemical gradient out of RBC $\rightarrow$ ↑ plasma HCO$_3^-$

- (5) DeoxyHb is a better buffer than OxyHb (Haldane Effect)
  - DeoxyHb (pKa 8.2) is a better buffer than OxyHb (pKa 6.6) because it is a weaker acid and dissociates to a greater extent
  - In tissue capillaries $\rightarrow$ OxyHb unloads O$_2$ to form DeoxyHb $\rightarrow$ This facilitates:
    - Uptake and buffering of extracellular H$^+$ (as reduced Hb; HHb) produced from hydration of tissue CO$_2$ and dissociation of H$_2$CO$_3$ $\rightarrow$ 30% Haldane effect
    - Uptake of tissue CO$_2$ (as carbamino compounds) $\rightarrow$ 70% Haldane effect
  - Thus, pH venous blood is only slightly more acidic than arterial blood in spite of large tissue CO$_2$ produced daily!

"Isohydric buffering" – For each mmol OxyHb that is reduced, 0.7mmol H$^+$ can be taken up by Hb, and 0.7mmol CO$_2$ can enter venous system without significantly changing pH

Note: Despite being a protein, Hb has 6x ↑ buffering capacity than protein proteins because
- (i) [Hb] is 2x of plasma protein
- (ii) Hb has 3x buffering capacity gram per gram that of plasma protein $\rightarrow$ due to 3x ↑ content of imidazole-containing histidine residues

**Protein buffer system:**
- Amino acid side chains can buffer H$^+$:
  - Most amino groups (pKa 9) and carboxyl groups (pKa 2) have pKa very far from physiological pH $\rightarrow$ Contributes little to buffering
  - Imidazole group on Histidine (pKa 6.8) has the closest pKa to physiological pH $\rightarrow$ Most important amino acid in proteins for buffering (esp in Hb; See above)
- Much more important buffer in ICF (cf. ECF) because $\rightarrow$ (i) [proteins] intracellularly are much ↑, and (ii) intracellular pH is more acidic (le. closer to pKa of amino acid moieties)

**Phosphate buffer system:**
- Phosphoric acid is a tribasic acid:
Main buffer intracellularly and in urine because → (i) [ ] are much ↑, and (ii) intracellular and urine pH are more acidic (i.e. closer to pKa of phosphate buffers)

Note: Despite biphosphate (H2PO4−) buffer having pKa very similar to physiological pH, it plays a minor role in extracellular buffering due to its low content in ECF and "closed" buffer system

- Important in “bone buffering” → CaPO4 acts as “Alkali reserve” → During prolonged acidosis, it solubilises in plasma to ↑ [PO4 3−]}

Acid-Base Homeostasis: Compensation

Respiratory compensation:
- This compensatory response is characterised by a very rapid and high capacity excretion of “respiratory acids” (~ 15,000 mmol H+/day as CO2) by the lungs → Nb. it does NOT involve excretion of “metabolic/fixed acids”
- Compensatory response involves two steps:
  o (1) PaCO2 is altered by changing minute ventilation
    Remember: PaCO2 = 0.83 x VCO2 / Va
  o (2) Change in PaCO2 normalises the [HCO3−]/PaCO2 ratio → Minimises changes in pH caused by acid-base disturbance
    Remember: pH = 6.1 + log ([HCO3−]/0.03xPCO2)

For example:
- During metabolic acidosis (↓ pH, ↑ [H+], ↓ [HCO3−]) → Induces hyperventilation → Lowers PaCO2 and normalises [HCO3−]/PaCO2 ratio
- During metabolic alkalosis (↑ pH, ↓ [H+], ↑ [HCO3−]) → induces hypoventilation → Increases PaCO2 and normalises [HCO3−]/PaCO2 ratio

- Regulation of respiratory compensation:
  o (i) Peripheral chemoreceptors (located in aortic and carotid bodies)
    ▪ Respond to ↓ arterial pH, ↓ PaO2 and ↑ PaCO2 → stimulate medullary ventilatory centres
    ▪ Nb. Minor role in responding to ↑ PaCO2 (only 20%) → BUT important for sensing acute changes in PaCO2
  o (ii) Central chemoreceptors (located in medulla)
    ▪ Responds to H+ in adjacent brain ECF (which is formed from CO2 traversing the BBB; and NOT plasma H+ which is insoluble in BBB) → stimulate medullary ventilatory centres
    ▪ Major role in responding to ↑ PaCO2 (80%)
    ▪ [HCO3−] in brain ECF equilibrates slowly (over 24 hrs) → alters chemoreceptor sensitivity in the event of prolonged acidosis

Note: MV ↑ 2L/min for every mmHg ↑ in PaCO2 from normal

Renal compensation:
- This compensatory response is characterised by a very slow (7-10 days) and low capacity 
excretion of “fixed/metabolic acids” (~ 70 mmol H+ /day) → normalises the [HCO₃⁻ ]/PaCO₂ ratio → Minimises changes in pH caused by acid-base disturbance

Note: This is the ONLY means of excreting “fixed acids” (Except for lactate → metabolised by liver)

- Renal regulation of acid-base balance involves tubular H⁺ secretion:
  o At least 4390 mmol H⁺ is actively secreted by kidneys each day:
    ▪ (i) Majority is used to reabsorb filtered HCO₃⁻ → 4320 mmol H⁺ is actively secreted by the tubular system each day to facilitate reabsorption of all 4320 mmol HCO₃⁻ filtered by the glomerulus (24 mmol HCO₃⁻ /L x 180 L/day = 4320 mmol) → this does not lead to net excretion of H⁺ in urine or addition of new HCO₃⁻ to blood
    ▪ (ii) Additional 70 mmol H⁺ is actively secreted to excrete “fixed/metabolic” acids to achieve a “net acid balance” of zero → 30 mmol/day as filtered buffers (titratable acids) and 40 mmol/day as manufactured buffers (NH₄⁺) → this leads to net excretion of H⁺ in urine (and addition of new HCO₃⁻ to blood)
  o During acidosis:
    ▪ (a) Tubular H⁺ secretion leads to all filtered HCO₃⁻ being reabsorbed
    ▪ (b) Excess H⁺ is secreted and lost in urine bound to non-absorbable buffers (filtered buffers and manufactured buffers) → results in an additional 300 mmol H⁺ excreted in urine per day (mainly bound to NH₄⁺) and an additional HCO₃⁻ added to blood
  o During alkalosis, excess plasma HCO₃⁻ (> 28 mmol/L) is excreted in urine by:
    ▪ (a) Loss of filtered HCO₃⁻ (Ie. not all is reabsorbed in tubular system)
    ▪ (b) Secretion of HCO₃⁻ from “type B intercalated cells” in CCD

Summary of “net acid excretion”:

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Acidosis</th>
<th>Alkalosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Titratable acids (mmol/day)</td>
<td>30</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>NH₄⁺ (mmol/day)</td>
<td>40</td>
<td>160</td>
<td>0</td>
</tr>
<tr>
<td>HCO₃⁻ (mmol/day)</td>
<td>0</td>
<td>0</td>
<td>80</td>
</tr>
<tr>
<td>NAE / “New” HCO₃⁻ added (mmol/day)</td>
<td>+70</td>
<td>200</td>
<td>-80</td>
</tr>
<tr>
<td>Urine pH</td>
<td>6.0</td>
<td>4.6</td>
<td>8.0</td>
</tr>
</tbody>
</table>

NAE = NH₄⁺ + “Titratable acids” – HCO₃⁻

Note: Filtered H⁺ at the glomerulus accounts for a very small % of excreted H⁺ → 36 nM x 180 L/day = 6.48 umol of excreted H⁺ per day

- Renal regulation of acid-base balance involves the following processes:
  o (1) Secretion of H⁺ associated with HCO₃⁻ reabsorption by renal tubules:
    ▪ Majority of HCO₃⁻ is reabsorbed by proximal tubular system → PCT (85%) and TAL (10%):
      ◦ High capacity and low gradient system → Because 3900 mmol H⁺ secreted/day (and 95% filtered HCO₃⁻ reabsorbed) BUT lowest urine pH achieved is only 7
      ◦ In the brush border membrane of PCT tubular luminal cells:
- H⁺ is secreted into the tubular lumen via apical Na⁺/H⁺ antiport → Secondarily active transport dependent on Na⁺ gradient generated by basolateral Na⁺/K⁺ ATPase
- This H⁺ then binds tubular luminal HCO₃⁻ filtered freely at the glomerulus to form H₂CO₃ → brush border CA then catalyses conversion of H₂CO₃ into CO₂ and H₂O, which are reabsorbed into tubular cells
- Within tubular cells throughout the tubular system:
  - H₂O and CO₂ reabsorbed from the tubular lumen is catalysed by intracellular CA into H₂CO₃, which dissociates into H⁺ and HCO₃⁻
  - HCO₃⁻ produced is reabsorbed into the peritubular capillary
  - H⁺ produced is then actively secreted back into tubular lumen where it can – (a) Aid in reabsorbing more HCO₃⁻, or (b) Participate in net acid excretion once all HCO₃⁻ has been bound

- Minority is reabsorbed by distal tubular system → DCT/CCD (5%):
  - Low capacity and high gradient system → Because 420 mmol H⁺ secreted/day (and 5% filtered HCO₃⁻ reabsorbed) BUT urine pH created is as low as 4.5
  - Within “type A intercalated cells”, CO₂ is hydrated to form H₂CO₃ (via intracellular CA) → dissociates into H⁺ and HCO₃⁻
  - H⁺ derived from this reaction is secrete H⁺ into the tubular lumen via apical H⁺ ATPase and H⁺/K⁺ ATPase (primary active transports) → controlled by aldosterone
  - HCO₃⁻ produced via this reaction is absorbed into peritubular capillary

Note – Type B intercalated cells in CCD → secrete HCO₃⁻ produced from IC dissociation of H₂CO₃ via a luminal Cl⁻/HCO₃⁻ exchanger → little importance because:
- (i) Very few type B intercalated cells (more type A cells in CD)
- (ii) Net tubular HCO₃⁻ handling is ALWAYS reabsorption (never secretion)

(2) Formation of titratable acidity:
Once all $\text{HCO}_3^-$ in tubular fluid are reabsorbed by $\text{H}^+$ secretion, excess $\text{H}^+$ secreted in tubular fluid are bound to “filtered buffers” in the distal tubular system (DCT and collecting ducts) and excreted in urine:

- (i) Mainly phosphate buffer system $\rightarrow$ $\text{H}^+$ combines 1°ly with $\text{HPO}_4^{2-}$ to form $\text{H}_2\text{PO}_4^-$ (as urine acidity $\sim 4.5 \rightarrow$ close to $\text{HPO}_4^{2-}$ buffer pKa of 6.8)
- (ii) Organic buffer systems (Eg. creatinine, sulphate, $\beta$-hydroxybutyrate)

Within distal tubular cells, $\text{CO}_2$ is hydrated to form $\text{H}_2\text{CO}_3$ (via intracellular CA) $\rightarrow$ dissociates into $\text{H}^+$ (which is secreted into tubular lumen via a secondarily active Na$^+$/H$^+$ anti-port $\rightarrow$ $\text{H}^+$ bind “filtered buffers”) and a newly formed $\text{HCO}_3^-$ (which is absorbed into peritubular capillary)

“Filtered buffers” are vital for excretion of “fixed acids” under normal conditions $\rightarrow$ Leads to excretion of 30 mmol of “fixed acids” per day

Note – They cannot be ↑ to excrete additional acid load during acidosis because:

- (i) Availability of filtered buffers cannot be easily ↑ cf. “manufactured buffers” (Ie. phosphate buffer amount is dependent on diet and PTH levels)
- (ii) Buffering capacity of filtered buffers maxed out at ↓ urine pHs

Aside: “Titratable acidity”
- Measured by the amount of alkali (as NaOH) that must be added to urine to return its pH to 7.4 (which is the pH of glomerular filtrate)
- Accounts for only a FRACTION of the $\text{H}^+$ secreted by the kidneys:
  - Accounts mainly for the $\text{H}^+$ that are bound to “filtered buffers” (phosphate and organic buffer systems) and excreted in urine
  - Does not account for $\text{HCO}_3^-$ buffer system $\rightarrow$ because $\text{H}_2\text{CO}_3$ is converted and reabsorbed as $\text{CO}_2$ and $\text{H}_2\text{O}$ $\rightarrow$ thus alters urine pH minimally
  - Ammonia buffer system contributes little to “titratable acidity” $\rightarrow$ because of high pKa of buffer system (9.1) (Ie. urine only titrated up to a pH of 7.4 and not any further)

- (3) Ammonia secretion:
  - Once all $\text{HCO}_3^-$ in tubular fluid are reabsorbed by $\text{H}^+$ secretion, excess $\text{H}^+$ secreted in tubular fluid are also bound to “manufactured buffers” in the distal tubular system (medullary collecting ducts) and excreted in urine
  - Ammoniagenesis $\rightarrow$ PCT tubular cells produce NH$_3$;
• Filtered glutamine is taken up by PCT tubular cells and deaminated via Glutaminase (which is upregulated with chronic acidosis) into NH$_4^+$ and HCO$_3^-$

• NH$_4^+$ is secreted into the tubular lumen via a Na$^+$/NH$_4^+$ antiport, while “newly formed” HCO$_3^-$ is reabsorbed into peritubular capillary blood

Ammonium cycling → In the TAL of LOH, 80% of tubular NH$_4^+$ is reabsorbed → this generates ↑ gradient of NH$_4^+$ in the medullary interstitium

Ammonium excretion → Later in the medullary CD:
• NH$_3$ diffuses from medullary interstitium into tubular cells of the CD, then into tubular fluid
• Within the tubular cells of the CD, H$^+$ and HCO$_3^-$ is formed by hydration of CO$_2$ using intracellular CA
• H$^+$ secreted into tubular fluid by Na$^+$/H$^+$ antiport → combines with tubular NH$_3$ to form NH$_4^+$ → “ion trapped” in tubular fluid

These “manufactured buffers” are vital for excretion of “fixed acids” under both:
• (a) Normal conditions → Accounts for 40 meq of H$^+$ excretion per day
• (b) Acidotic conditions → Can excrete an additional 300 mmol H$^+$ per day due to:
  o (i) ↑ transfer at low urine pH due to high pKa 9.2 of buffer system (Ie. allows additional H$^+$ to be excreted even when tubular fluid has reached maximal acidity)
  o (ii) ↑ production of glutamine (very slow process)
Only a small amount of $H^+$ can be excreted in its free form because active transport of $H^+$ secretion is inhibited at high urinary $[H^+]$. Thus, the lowest urinary pH achieved is 4.4.

As a result, $H^+$ secretion and excretion in urine is dependent on binding to "urinary buffers":

1. $HCO_3^-$ buffer ($pK_a = 6.1$),
2. $HPO_4^{2-}$ buffer ($pK_a = 6.8$), and
3. $NH_3$ buffer ($pK_a = 9.1$)

In the absence of these urinary buffer systems, the urine pH of 4.4 would be reached very rapidly, and any further $H^+$ secretion would cease.

### Clinical Effects of Acid-Base Changes:

<table>
<thead>
<tr>
<th>System</th>
<th>Acidosis</th>
<th>Alkalosis</th>
</tr>
</thead>
</table>
| CVS    | - Direct −ve inotropic effect:  
- Due to ↓ slow inward $Ca^{2+}$ current and ↓ $Ca^{2+}$ release from SR  
- Initially opposed by medullary catecholamine response → until pH 7.2 then −ve inotropy  
- ↑ SNS activity (medullary catecholamine release):  
- Offsets −ve inotropy  
- BUT ↑ cardiac arrhythmias, ↑ SVR and renal/splanchnic vasoconstriction  
- Cardiac arrhythmias:  
- Due to ↓ IC [K+] (↑ RMP of pacemaker cells) and ↑ adrenal medulla catecholamine release  
- Vascular effect  
- Mild acidosis → ↑ SVR and renal/splanchnic vasoconstriction (due to medullary SNS response)  
- ↑ acidosis → Vasodilation (skin, skeletal muscle, heart) and ↓ SVR  
- Pulmonary vasoconstriction → HTN | - ↑ coronary VC and ↑ SVR |
| Respiratory | ↑ MV → ↑ response with respiratory acidosis cf. metabolic acidosis b/c $CO_2$ more permeable than $H^+$ at BBB  
- Bronchodilation (due to hypercapnoea)  
- Right shift of $O_2$ HDC → ↑ $O_2$ tissue delivery | - Opposite effects to acidosis |
| CNS | Impairs LOC due to changes in CBF and ICP | Epilepsy |
| GIT | - Splanchnic vasoconstriction  
- ↓ GIT motility | |
| Electrolyte | ↑ free ionised serum $Ca^{2+}$ → due $H^+$ competing for −ve binding on albumin (chronically, $2^o$ to $Ca^{2+}$ mobilisation from bone)  
- ↑ serum $K^+$ → EC $H^+$ exchanged for IC $K^+$ (0.6 mmol ↑ [K+] per 0.1 ↓ pH) | - Opposite effects to acidosis |
(d) To explain the principles of blood gas and acid-base analysis.

(e) To interpret blood gas analysis and its management in clinical situations.

Definitions in Acid-Base Disorders:

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidaemia</td>
<td>Arterial blood pH &lt; 7.36 and [H+] &gt; 44 nmol/L</td>
</tr>
<tr>
<td>Alkalaemia</td>
<td>Arterial blood pH &gt; 7.44 and [H+] &lt; 36 nmol/L</td>
</tr>
<tr>
<td>Acidosis</td>
<td>Abnormal process that tends to ↓ blood pH (i.e., cause acidaemia) if there are no secondary changes in the response to the primary disease</td>
</tr>
<tr>
<td>Alkalosis</td>
<td>Abnormal process that tends to ↑ blood pH (i.e., cause alkalaemia) if there are no secondary changes in the response to the primary disease</td>
</tr>
</tbody>
</table>

Primary aetiological disorder can be either:

- Respiratory: Changes in CO₂ → lead to changes in respiratory acid (H₂CO₃)
- Metabolic: Changes in fixed/metabolic acids or HCO₃⁻

Primary aetiological disorder can also be either:

- Simple: Single primary aetiological acid-base disorder present
- Mixed: > 2 primary aetiological disorders present simultaneously

Assessment of Acid-Base Disorders:

- Assessing an acid-base disorder involves determining the (i) type of primary disorder (acidaemia vs alkalaemia; respiratory vs metabolic; simple vs mixed), (ii) degree of compensation present, and (iii) possible aetiology of the disorder

- This requires information derived from:
  - (1) History and physical examination
  - (2) ABG
    - (a) pH
      - Measured directly using pH electrodes (See below)
      - Assesses acidity or alkalinity of blood
    - (b) PaCO₂
      - Measured directly using CO₂ electrode (See below)
      - Assesses respiratory component of pH shift
    - (c) [HCO₃⁻]
      - Assesses metabolic component of pH shift
      - Measured two ways:
        - (i) Plasma HCO₃⁻ – Measured indirectly with Henderson-Hasselbach equation (using known pH and PaCO₂ measurements) → thus levels inaccurate since pKa used for H₂CO₃ does not account for fluctuations in temperature and PaCO₂
        - (ii) Standard HCO₃⁻ – Defined as [HCO₃⁻] in whole blood which is fully oxygenated with PaCO₂ 40 mmHg and temp 37°C (normally 24 +/- 2 mmol/L) → preferred over plasma HCO₃⁻
    - (d) Base excess (or deficit)
      - Defined as amount of strong acid or base required to titrate fully saturated whole blood at 37 °C and PaCO₂ 40 mmHg to a pH of 7.4 (normally 0 +/- 2 mEq/L)
- Buffer base – Sum of buffer anions [ ] in blood (Hb, HCO₃⁻, protein, phosphate) in fully oxygenated blood (normally 45-60 mmol/L)
- Base excess – Increase in buffer base → indicates ↑ buffering capacity (Ie. due to ↓ metabolic acids or ↑ in buffer systems)
- Base deficits – Decrease in buffer base → indicates ↓ buffering capacity (Ie. due to ↑ metabolic acids or ↓ buffer systems)

• Can be also used to measure metabolic component of pH shift → preferred over plasma HCO₃⁻

• Assumptions:
  o (i) When pH of blood is normal, ratios and total [ ] of non-carbonic buffers are normal
  o (ii) Blood behaves as simple HCO₃⁻ solution (Ie. controlled by bicarbonate-carbonic acid buffer system) → so all buffering (and changes in blood pH/[H⁺]) is achieved by changes in HCO₃⁻ levels → thus changes in [HCO₃⁻] reflects amount of acid/base added to blood
  o (iii) It is NOT affected by respiratory acid-base disturbances → because changes in PaCO₂ involves equal changes in plasma levels of H⁺ and HCO₃⁻ from H₂CO₃
• Clinical limitations:
  o (i) It is an in vitro system with a set of assumptions
  o (ii) It does not account for extravascular buffers or interactions of blood buffers with ISF/ICF buffers
  o (iii) Assess only blood buffers (33% of total body buffering capacity)
  o (iv) Tends to overestimate acid-base changes of whole body

- (e) PaO₂
  • Measured directly using Clark electrode (See below)
  • Assesses whether patient is hypoxaemic and has potential respiratory disease (Ie. ↑ A-a PO₂ gradient)
  • When FiO₂ is not known (Ie. assess if breathing supplemental O₂) → utilise Alveolar gas equation to determine PAO₂ → if PaO₂ > PAO₂, likely that patient is using supplemental O₂
- (f) Temperature
  • With ↓ temperature:
    o (i) ↑ gas solubility (CO₂ and O₂) → ↓ PaCO₂ (↓ 4.5% per ↓ 1°C) and ↓ PaO₂
    o (ii) ↑ pH
      • Neutral H₂O: pH ↑ 0.017 unit per ↓ 1°C
      • Blood: pH ↑ 0.015 unit per ↓ 1°C (Rosenthal correction factor) → Different from neutral H₂O due to imidazole moieties of histidine in Hb
  • Consequence → Assessment of blood at 37°C from hypothermic patient can lead to falsely ↑ PO₂ and PCO₂ and falsely ↓ pH → thus above “correction factors” are applied to avoid this
Note – There are two means to correct for temperature changes:

(i) α-stat hypothesis
- Refers to the theory that the degree of ionisation of imidazole groups should remain constant despite changes in temperature whilst keeping CO₂ stores constant (i.e. pH changes with temp)
- So with ↓ temp → pKa of imidazole groups on proteins will ↓ → % unprotonated imidazole groups remain constant → thus, no change in CO₂ stores
- Blood sample is heated to 37°C and is interpreted against values at that temperature irrespectively of what the patient’s temperature is

(ii) pH-stat hypothesis
- Refers to the theory that pH should remain constant despite changes in temperature
- To overcome ↑ CO₂ with ↑ temp → CO₂ is added to system to maintain a constant PCO₂ ~ 40mmHg → thus, there is an overall ↑ in CO₂ stores
- Blood sample is measured against normalised values at 37°C regardless of what the patient’s temperature is (as there is no change in pH with temp change)

- (g) FiO₂
- (h) SaO₂

- (3) Biochemistry laboratory results → used to determine:
  - (a) Anion gap (AG):
    - AG represents all the unmeasured anions in plasma (sulphates, phosphates, organic acids, proteins) → normally 12 +/-2 mmol/L
    - \[\text{AG} = [\text{Na}^+ + \text{K}^+] - [\text{Cl}^- + \text{HCO}_3^-]\]
    - Nb. An AG arises because routine clinical electrolyte measurements include most cations (Na⁺ and K⁺) but only some anions (Cl⁻ and HCO₃⁻) → thus, several anions in plasma remain unmeasured. Since the law of electroneutrality states that sum of +ve charges is balanced by sum of –ve charges, the AG is the “apparent” difference between the total measured cation [ ] and total measured anion [ ]
  
- (b) Osmolar gap (OG):
  - OG is the difference between measured and calculated serum osmolality (normally < 15 mOsm/kg)
  - Assist in differentiating causes elevated AG metabolic acidosis (i.e. ↑ with presence of circulating intoxicants, such as methanol)
  - \[\text{OG} = \text{“Measured” serum osmolality} - \text{“Calculated” serum osmolality}\]
  - Where “calculated” serum osmolality = 2x[Na⁺] + [urea] + BSI.
Types of Acid-Base Disturbances:

(I) Respiratory acidosis:
- Defined as a primary acid-base disorder due to rise in PaCO₂ to level higher than expected (i.e., ↑H₂CO₃)
- Causes:
  o Alveolar hypoventilation (i.e., ↓MV, ↑dead space)
  o ↑inspired PCO₂ (Eg. CO₂ absorber saturated)
  o ↑CO₂ production (Eg. fever, TTX, MH)
- Buffering/compensatory response:
  o Acutely → Buffering (mainly IC buffers) and respiratory correction (↑MV)
  o Chronic:
    ▪ Renal compensation by retention of HCO₃⁻ – ↑PaCO₂ causes proximal tubular cells to ↑H⁺ secretion → ↑production of HCO₃⁻
    ▪ ↓plasma Cl⁻ – Net renal H⁺ excretion causes Cl⁻ to maintain electroneutrality → leads to +ve BE
- ABG findings:

<table>
<thead>
<tr>
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</tr>
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<tbody>
<tr>
<td>pH</td>
<td>↓ (expect 7.25 @ PaCO₂ 60; 7.15 @ PaCO₂ 80)</td>
<td>Normalises (but &lt; 7.4)</td>
</tr>
<tr>
<td>PaCO₂</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>BE</td>
<td>Normal</td>
<td>+ve</td>
</tr>
</tbody>
</table>
| HCO₃⁻ | ↑ (expect compensation if [HCO₃⁻])         | ↑ (expect compensation if [HCO₃⁻] ↑ by 4)
(II) Respiratory alkalosis:
- Defined as a primary acid-base disorder due to fall in PaCO₂ to a level lower than expected (Ie. ↓ H₂CO₃)
- Causes:
  o Invariably due to alveolar hyperventilation (Ie. ↑ MV, ↓ DS)
  o ↓ CO₂ production (Eg. GA, hypothermia)
- Buffering/compensatory response:
  o Acutely → Buffering (mainly IC buffers) and respiratory correction (↓ MV → but limited by need to maintain oxygenation!)
  o Chronic → Renal compensation by ↑ HCO₃⁻ excretion and ↓ NH₄⁺ excretion (Ie. net H⁺ retention/HCO₃⁻ loss) → -ve BE and ↓ serum HCO₃⁻
- ABG findings:

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<tbody>
<tr>
<td>pH</td>
<td>↑ (expect 7.5 @ PaCO₂ 30; 7.6 @ PaCO₂ 40)</td>
<td>Normalises (but &gt; 7.4)</td>
</tr>
<tr>
<td>PaCO₂</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
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<td>Normal</td>
<td>-ve</td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>↓ (expect compensation if [HCO₃⁻])</td>
<td>↓ (expect compensation if [HCO₃⁻] ↓ by 5 mmol/L for each 10 mmHg ↓ PaCO₂ above 40 mmHg)</td>
</tr>
</tbody>
</table>

- Treatment → Correct underlying cause

(III) Metabolic acidosis:
- Defined as a primary acid-base disorder that causes plasma HCO₃⁻ to fall to a level lower than expected due to either (i) an increase in metabolic/fixed acids, and/or (ii) loss of bases in blood
- Causes:
  o ↑ AG → Due to replacement of HCO₃⁻ with fixed/metabolic acids (which are unmeasured anions) → MUD PILES
  o Normal AG → Usually associated with hyperchloraemia → GI losses (diarrhoea, pancreatic fistula, external drainage of pancreatic/biliary secretions, uretero-enterostomy, obstructed ileal conduit), RTA, renal interstitial disease, mineralocorticoid deficiency, infusion of HCl/NH₄Cl or CAi (acetazolamide)
  o ↓ AG → Hypoproteinaemic states
- Buffering/compensatory response:
  o Acutely:
    ▪ Buffering (60% IC buffers; 40% EC buffers)
    ▪ Respiratory compensation
      • Initially – ↓ pH triggers peripheral chemoreceptors → hyperventilation → ↓ PaCO₂ to partly normalise pH
      • BUT this ↓ brain ECF [H⁺] and causes central chemoreceptors to limit the ↑ in MV
      • Full effect of respiratory compensation requires 12-24 hrs – HCO₃⁻ equilibrates across BBB and brain ECF [H⁺] ↑ → inhibition on MV by central chemoreceptors gradually removed
      • Nb. Respiratory compensation CANNOT excrete fixed acids!
  o Chronic → Renal compensation by excreting excess acid anions (equivalent to reabsorption of HCO₃⁻/excretion of H⁺)
- ABG findings:

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<tbody>
<tr>
<td>pH</td>
<td>↓</td>
<td>Normalises (but &lt; 7.4)</td>
</tr>
</tbody>
</table>
| PaCO₂| Normal| ↓ (expect compensation if PaCO₂ is
BE -ve ↓
HCO₃⁻ -ve ↓↓

- Treatment:
  o (i) Eliminate causative factor
  o (ii) IV NaCl → allows kidneys to excrete sufficient HCl to correct acidosis (if acidaemia is not affecting C.O.)
  o (iii) IV HCO₃⁻ (if acidaemia depressing C.O. → avoid vicious cycle of worsening CVS depression with increasing lactic acidosis)
  o (iv) Dialysis

*(IV) Metabolic alkalosis:*
- Defined as a primary acid-base disorder that causes plasma HCO₃⁻ to rise to a level higher than expected due to either (i) an gain in bases, and/or (ii) loss of metabolic/fixed acids in blood
- Causes:
  o Loss of acids → Renal (hyperaldosteronism, Cushing’s, thiazide diuretics, severe hypokalaemia, hypomagnesiusma, hypercalcaemia) or GIT (NG suctioning, severe vomiting)
  o Increased base intake → NaHCO₃ administration, metabolic conversion of exogenous organic ions (Eg. Lactate)
- Buffering/compensatory response:
  o Acutely → Buffering (70% EC buffers; 30% IC buffers) and respiratory compensation (↓ MV)
  o Chronic → Renal compensation by excretion of excess HCO₃⁻/retention of H⁺
- ABG findings:

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<td>↑↑↑ (but &gt; 7.4)</td>
</tr>
<tr>
<td>PaCO₂</td>
<td>Normal</td>
<td>↑ (expect compensation if PaCO₂ is 0.7x[HCO₃⁻] + 20)</td>
</tr>
<tr>
<td>BE</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>↑↑↑</td>
<td>↑↑↑</td>
</tr>
</tbody>
</table>

- Treatment:
  o (i) Eliminate causative factor
  o (ii) Replace volume deficit with NaCl → allows excretion of NaHCO₃
  o (iii) IV NH₄Cl (if very severe alkalosis or poor renal/cardiac function)
  o (iv) IV HCl avoided due to very low pH and need for administration by CVC only
  o (v) CAi (Eg. acetazolamide)
  o (vi) Spironolactone (to ↑ K⁺)
Aside: Measurement of pH, PCO\textsubscript{2} and PO\textsubscript{2} from ABG

**Measurement of pH:** pH electrode (Glass-Calomel electrode)

- Consists of two ion-selective electrodes:
  - (i) Measuring electrode
    - Ag/AgCl surrounded by buffer solution in glass casing \( \rightarrow \) glass bulb at electrode tip made of special pH-sensitive glass (-vely charged glass that is permeable to $H^+$) that is in direct contact with arterial blood sample
    - $H^+$ diffuses from blood sample through the glass membrane into buffer solution \( \rightarrow \) buffer solution allows a pH (and $[H^+]$) gradient to form across the glass membrane \( \rightarrow \) produces an electrical potential difference that is dependent on the pH (and $[H^+]$) gradient
  - (ii) Reference electrode
    - Hg/Hg\textsubscript{2}Cl\textsubscript{2} (calomel) surrounded by saturated 0.1 M KCl solution ("salt bridge") that is separated from the arterial blood sample via a semi-permeable membrane
    - Maintains constant potential despite changes in arterial pH
- These two electrodes are connected via blood to create an electrical circuit \( \rightarrow \) electrical potential difference produced is proportional to pH (and $[H^+]$) gradient $\rightarrow$ 61.5 mV/pH unit
- It has an accuracy of +/- 0.005 pH units
- Issues:
  - (i) Both electrodes must be:
    - Kept at 37°C \( \rightarrow \) due to temperature-dependent changes in pH (acid/bases dissociate at \( \uparrow \) temperatures \( \rightarrow \) so pH $\downarrow$ 0.015 unit per \( \uparrow \) 1°C $\rightarrow$ Rosenthal factor) and solubility of gases (Ie. CO\textsubscript{2})
    - Kept clean (esp free from protein and cellular deposits)
    - Calibrated with 2x PO\textsubscript{4}\textsuperscript{3-} buffer solutions of known pH
  - (ii) Delicate plastic membrane $\rightarrow$ damage leads to inaccurate results (due to protein or microorganism contamination of electrodes)

**Measurement of PCO\textsubscript{2}: CO\textsubscript{2} electrode (Severinghaus electrode)**

- Modified pH electrode $\rightarrow$ two ion-selective electrodes contained in a CO\textsubscript{2}-permeable plastic membrane (not permeable to liquids and solids, such as proteins) that separates them from blood sample:
  - (i) $H^+$-sensitive glass electrode $\rightarrow$ covered in nylon mesh and coated in thin film of NaHCO\textsubscript{3} solution
  - (ii) Ag/AgCl reference electrode
- CO₂ diffuses from blood sample across plastic membrane into the NaHCO₃-coated nylon mesh → CO₂ hydrated to form H⁺ and HCO₃⁻ → glass electrode measures change in H⁺ (and pH) in NaHCO₃ solution, which is proportional to changes in CO₂ tension
- It has an accuracy of +/- 1 mmHg
- Issues:
  - (i) Slow response time (2-3 mins) → because CO₂ needs to diffuse across membrane and react with H₂O to form H⁺ and HCO₃⁻ (Nb. Can be accelerated by addition of CA)
  - (ii) Delicate plastic membrane → damage leads to inaccurate results
  - (iii) Both electrodes must be kept at 37°C, clean (i.e. free from protein deposits) and calibrated with buffer solutions of known PCO₂

**Measurement of PO₂: O₂ electrode (Clark electrode)**

- Cathode (Pt wire in glass rod) and anode (Ag wire in AgCl gel) placed in KCl solution → this is wrapped in an O₂-permeable plastic membrane (not permeable to liquids and solids, such as proteins) that separates electrodes from blood sample
- O₂ diffuses across membrane from blood sample to equilibrate with electrolyte solution → potential difference of 600 mV is then applied to electrodes → causes an electron flow from anode to cathode as per the following reactions:
  - At cathode: O₂ + 2 H₂O + 4e⁻ → 4 OH⁻
  - At anode: Ag + Cl⁻ → AgCl + e⁻
  - In solution: KCl + OH⁻ → KOH + Cl⁻
- Only at an applied voltage of 600 mV → current generated in circuit is directly proportional to PO₂ (i.e. linear relationship)
- It has an accuracy of +/− 2 mmHg
- Issues:
  o (i) Electrodes → must be kept at 37°C, clean and contaminant-free (i.e. free from protein deposits and O₂-consuming cells/microorganisms), and calibrated with solutions of known PO₂ (with N₂ as zero-point)
  o (ii) Delicate plastic membrane → damage leads to inaccurate results (due to protein or microorganism contamination of electrodes)
  o (iii) Halothane can produce falsely ↑ PO₂ readings → avoid this issue by using membrane impermeable to it
  o (iv) Avoid delay in sample analysis → cells consume O₂ which causes falsely lower PO₂ (Nb. store sample in ice to retard cell O₂ consumption)
  o (v) Applied voltage must be 600 mV → this is b/c:
    ■ (a) Calibration curve of current vs. PO₂ is linear
    ■ (b) Relationship of current and voltage is non-linear at a given PO₂, EXCEPT within a plateau region (400-800 mV) → in this range, current flow for a given PO₂ is not altered by small changes in applied voltage (i.e. less likely that fluctuations in applied voltage will bias PO₂ readings)